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Signed, this 31st day of January 2008,

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FEDERAL REPUBLIC OF GERMANY

Certificate of Priority for Filing of a Patent Application

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Munich, 12th February 2004
On behalf of the President of the German Patent and Trade Mark Office

(signature)

Hemus

A process for the production of L-amino acids using strains of the Enterobacteriaceae family

The present invention relates to a process for the production of L-amino acids, in particular L-threonine, using strains of the Enterobacteriaceae family in which the galP gene is overexpressed.

Prior art

L-amino acids, in particular L-threonine, are used in human medicine and in the pharmaceutical industry, in the foodstuffs industry and very particularly in animal nutrition.

It is known that L-amino acids can be prepared by the fermentation of strains of Enterobacteriaceae, in particular Escherichia coli (E. coli) and Serratia marcescens. As a result of the great importance of this, efforts are constantly made to improve the method of production. Process improvements may relate to fermentation engineering measures such as e.g. stirring and supplying with oxygen, or to the composition of the nutrient media such as e.g. the sugar concentration during fermentation, or to working up to the product form by e.g. ion-exchange chromatography, or the intrinsic performance characteristics of the microorganism itself.

The methods of mutagenesis, selection and mutant choice are used to improve the performance characteristics of these microorganisms. In this way, strains are obtained which are resistant to antimetabolites such as e.g. the threonine analogon α-amino-β-hydroxyvaleric acid (AHV) or auxotrophic for regulatorily important metabolites and which produce L-amino acids such as e.g. L-threonine.

For some time now, the methods of recombinant DNA engineering have also been used for the strain-improvement of L-amino acid-producing strains of the Enterobacteriaceae

family, by amplifying individual amino acid biosynthesis genes and investigating the effect of this on production. A summary of the information relating to the cellular biology and molecular biology of Escherichia coli and Salmonella can be found in Neidhardt (ed): Escherichia coli and Salmonella, Cellular and Molecular Biology, 2nd edition, ASM Press, Washington, D.C., USA,(1996).

Object of the invention

The inventors have made the object the provision of new measures for the improved fermentative production of L-amino acids, in particular L-threonine.

Description of the invention

The invention provides a process for the fermentative production of L-amino acids, in particular L-threonine, using microorganisms from the Enterobacteriaceae family, in particular those which already produce L-amino acids and in which the nucleotide sequence coding for the galP gene is overexpressed.

The gene product coded by the galP gene is known in the prior art, inter alia, as "galactose proton symporter" or "galactose permease".

Wherever L-amino acids or amino acids are mentioned in the following, this is intended to mean one or more amino acids, including their salts, chosen from the group comprising L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophane and L-arginine. L-threonine is particularly preferred.

The term "overexpression" describes in this connection the increase in intracellular activity or concentration of one or more enzymes or proteins in a microorganism which are

coded by the corresponding DNA, by for example increasing the copy number of the gene or genes by at least one (1) copy or using a strong promoter and optionally combining these measures.

As a result of the overexpression measures the activity or concentration of the corresponding protein is generally increased by at least 10%, 25%, 50%, 75%, 100%, 150%, 200%, 300%, 400% or 500%, at most up to 1000% or 2000%, with respect to the activity or concentration of the protein in the starting microorganism.

The invention provides a process for the production of L-amino acids by the fermentation of recombinant microorganisms from the Enterobacteriaceae family, characterised in that

a) the microorganisms producing the desired L-amino acid, in which the galP gene or nucleotide sequences coding for galactose permease is/are overexpressed, is cultured in a medium under conditions in which the desired L-amino acid is enriched in the medium or in the cells, and

b) the desired L-amino acid is isolated, wherein all or some (>0 to 100 %) of the constituents of the fermentation broth and/or the biomass optionally remain in the isolated product or are completely removed.

The recombinant microorganisms which are also provided by the present invention, can produce L-amino acids from glucose, saccharose, lactose, fructose, maltose, molasses, optionally starch, optionally cellulose, or from glycerol and ethanol. They are representatives of the Enterobacteriaceae family chosen from the genera Escherichia, Erwinia, Providencia and Serratia. The genera Escherichia and Serratia are preferred. In the case of the genus Escherichia, the species Escherichia coli is

mentioned in particular and in the case of the genus Serratia, the species Serratia marcescens is mentioned in particular.

Suitable strains of the genus Escherichia, in particular those producing L-threonine, in particular of the species Escherichia coli are, for example

- Escherichia coli H4581 (EP-A 0 301 572)
- Escherichia coli KY10935 (Technical Research Laboratories 61(11):1877-1882 (1997)
- Escherichia coli VNIIgenetika MG442 (US-A-4278,765)
- Escherichia coli VNIIgenetika M1 (US-A-4,321,325)
- Escherichia coli VNIIgenetika 472T23 (US-A-5,631,157)
- Escherichia coli BKIIM B-3996 (US-A-5,175,107)
- Escherichia coli kat 13 (WO 98/04715)
- Escherichia coli KCCM-10132 (WO 00/09660)

Suitable strains of the genus Serratia, in particular those producing L-threonine, in particular of the species Serratia marcescens are, for example

- Serratia marcescens HNr21 (Applied and Environmental Microbiology 38(6): 1045-1051 (1979))
- Serratia marcescens TLr156 (Gene 57(2-3): 151-158 (1987))
- Serratia marcescens T-2000 (Applied Biochemistry and Biotechnology 37(3): 255-265 (1992))

L-threonine-producing strains from the Enterobacteriaceae family preferably possess, inter alia, one or more of the genetic or phenotypical features chosen from the group:

resistance to α-amino-β-hydroxyvaleric acid, resistance to thialysine, resistance to ethionine, resistance to α-methylserine, resistance to diaminosuccinic acid, resistance to α-aminobutyric acid, resistance to borrelidine, resistance to cyclopentanecarboxylic acid, resistance to rifampicin, resistance to valine analogues such as, for example, valinehydroxamate, resistance to purine analogues such as, for example, 6-dimethyl-aminopurine, a requirement for L-methionine, optionally a partial and compensatable requirement for L-isoleucine, a requirement for meso-diaminopimelic acid, auxotrophy with respect to threonine-containing dipeptides, resistance to L-threonine, resistance to threonine raffinate, resistance to L-homoserine, resistance to L-lysine, resistance to L-methionine, resistance to L-glutamic acid, resistance to L-aspartate, resistance to L-leucine, resistance to L-phenylalanine, resistance to L-serine, resistance to L-cysteine, resistance to L-valine, sensitivity towards fluoropyruvate, defective threonine dehydrogenase, optionally the ability to utilise saccharose, overexpression of the threonine operon, overexpression of homoserine dehydrogenase I-aspartate kinase I, preferably the feedback resistant form, overexpression of homoserine kinase, overexpression of threonine synthase, overexpression of aspartate kinase, optionally the feedback resistant form, overexpression of aspartate semialdehyde dehydrogenase, overexpression of phosphoenolpyruvate carboxylase, optionally the feedback resistant form, overexpression of phosphoenolpyruvate synthase, overexpression of transhydrogenase, overexpression of the RhtB gene product, overexpression of the RhtC gene product, overexpression of the YfiK gene product, overexpression of a pyruvate carboxylase, and attenuation of acetic acid formation.

It was found that microorganisms of the Enterobacteriaceae family produce L-amino acids, in particular L-threonine, in an improved manner after overexpression of the galP gene.

The nucleotide sequences of the genes from Escherichia coli are part of the prior art (see following text citations) and can also be obtained from the genome sequence for Escherichia coli published by Blattner et al. (Science 277: 1453-1462 (1997)).

The protein for which the galP gene codes is described, inter alia, by the following data:

Name:	sugar transporter, galactose proton symporter, galactose permease
Function:	as an integral membrane protein, symport of 2-deoxy-D-galactose and a proton into cells
Reference:	Macpherson et al.; The Journal of Biological Chemistry 258(7): 4390-4396 (1983), Venter et al.; The Biochemical Journal 363(Pt 2): 243-252 (2002)
Accession No.:	AE000377

Galactose permease in Salmonella typhimurium is described, inter alia, in the following references:
Postma; Journal of Bacteriology 129(2): 630-639 (1977);
Nagelkerke and Postma; Journal of Bacteriology 133(2): 607-613 (1978).

The nucleic acid sequences can be obtained from the databank at the National Center for Biotechnology Information (NCBI) at the National Library of Medicine (Bethesda, MD, USA), the nucleotide sequence databank at the European Molecular Biologies Laboratories (EMBL, Heidelberg, Germany and Cambridge, UK) or from the DNA databank of Japan (DDBJ, Mishima, Japan).

The genes described in the cited literature references can be used in accordance with the invention. Furthermore, alleles of the genes can be used where these are produced by degeneracy of the genetic code. The use of endogenous genes is preferred.

"Endogenous genes" or "endogenous nucleotide sequences" are understood to be the genes or alleles or nucleotide sequences present in the population of a species.

In order to achieve an overexpression, the copy number of the corresponding genes can be increased, or the promoter and regulation region or the ribosome binding site which is located upstream of the structural gene can be mutated. Expression cassettes which are incorporated upstream of the structural gene act in the same way. By using inducible promoters, it is also possible to increase expression during the course of fermentative production of L-amino acids. Expression is also improved by measures to prolong the lifetime of the m-RNA. Furthermore, enzyme activity is likewise enhanced by preventing degradation of the enzyme protein. The genes or gene constructs may either be present in extrachromosomally replicating plasmids with different copy numbers or be integrated and amplified in the chromosome. Alternatively, furthermore, overexpression of the relevant genes may be achieved by modifying the composition of the medium and culture management.

A person skilled in the art can find instructions for this, inter alia, in Chang and Cohen (Journal of Bacteriology 134: 1141-1156 (1978)), in Hartley and Gregori (Gene 13: 347-353 (1981)), in Amann and Brosius (Gene 40: 183-190 (1985)), in de Broer et al. (Proceedings of the National Academy of Sciences of the United States of America 80: 21-25 (1983)), in LaVallie et al. (BIO/TECHNOLOGY 11: 187-193 (1993)), in PCT/US97/13359, in Llosa et al. (Plasmid 26: 222-224 (1991)), in Quandt and Klipp (Gene 80: 161-169 (1989)), in Hamilton et al. (Journal of Bacteriology 171:

4617-4622 (1989)), in Jensen and Hammer (Biotechnology and Bioengineering 58: 191-195 (1998)) and in well-known textbooks on genetics and molecular biology.

Plasmid vectors which can be replicated in Enterobacteriaceae, such as e.g. cloning vectors derived from pACYC184 (Bartolomé et al.; Gene 102: 75-78 (1991)), pTrc99A (Amann et al.; Gene 69: 301-315 (1988)) or pSC101-derivates (Vocke and Bastia; Proceedings of the National Academy of Sciences USA 80(21): 6557-6561 (1983)) can be used. In a process according to the invention, a strain transformed with a plasmid vector may be used, wherein the plasmid vector contains at least one nucleotide sequence coding for galactose permease.

The expression transformation is understood to be the acceptance of an isolated nucleic acid by a host (microorganism).

It is also possible to convert mutations which affect expression of the particular gene by sequence exchange (Hamilton et al.; Journal of Bacteriology 171: 4617-4622 (1989)), conjugation or transduction in different strains.

More detailed explanations of concepts used in genetics and molecular biology can be found in well-known textbooks of genetics and molecular biology, such as for example the textbook by Birge (Bacterial and Bacteriophage Genetics, 4th ed., Springer Verlag, New York (USA), 2000) or the textbook by Stryer (Biochemistry, 3rd ed., Freeman and Company, New York (USA), 1988) or the manual by Sambrook et al. (Molekular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor (USA), 1989).

Furthermore, it may be advantageous for the production of L-amino acids, in particular L-threonine, using strains of the Enterobacteriaceae family, in addition to overexpressing the galP gene, to overexpress one or more

enzymes in the well-known threonine biosynthesis pathway or enzymes from anoplerotic metabolism or enzymes for the production of reduced nicotinamide-adenine dinucleotide phosphate or enzymes from glycolysis or PTS enzymes or enzymes from sulfur metabolism. The use of endogeneous genes is generally preferred.

Thus, for example, one or more genes chosen from the following group can be simultaneously enhanced, in particular overexpressed

- the thrABC operon coding for aspartate kinase, homoserine dehydrogenase, homoserine kinase and threonine synthase (US-A-4,278,765),
- the pyc gene from Corynebacterium glutamicum coding for pyruvate carboxylase (WO 99/18228),
- the pps gene coding for phosphoenolpyruvate synthase (Molecular and General Genetics 231(2): 332-336 (1992)),
- the ppc gene coding for phosphoenolpyruvate carboxylase (Gene 31: 279-283 (1984)),
- the pntA and pntB genes coding for transhydrogenase (European Journal of Biochemistry 158: 647-653 (1986)),
- the rhtB gene which imparts homoserine resistance (EP-A-0 994 190),
- the mqo gene coding for malate:quinone oxidoreductase (WO 02/06459),
- the rhtC gene which imparts threonine resistance (EP-A-1 013 765),
- the thrE gene from Corynebacterium glutamicum coding for threonine export protein (WO 01/92545),

- the gdhA gene coding for glutamate dehydrogenase (Nucleic Acids Research 11: 5257-5266 (1983); Gene 23: 199-209 (1983)),
- the hns gene coding for DNA binding protein HLP-II (WO 03/004671),
- the pgm gene coding for phosphoglucomutase(WO 03/004598),
- the fba gene coding for fructose biphosphate aldolase (WO 03/004664),
- the ptsH gene from the ptsHIcrr operon coding for phosphohistidine protein hexose phosphotransferase in the phosphotransferase system PTS (WO 03/004674),
- the ptsI gene from the ptsHIcrr operon coding for enzyme I in the phosphotransferase system PTS (WO 03/004674),
- the crr gene from the ptsHIcrr operon coding for the glucose-specific IIA component in the phosphotransferase systems PTS (WO 03/004674),
- the ptsG gene coding for the glucose-specific IIBC component (WO 03/004670),
- the lrp gene coding for the regulator in the leucine regulon (WO 03/004665),
- the csrA gene coding for the global regulator Csr (Journal of Bacteriology 175: 4744-4755 (1993)),
- the fadR gene coding for the regulator in the fad regulon (Nucleic Acids Research 16: 7995-8009 (1988)),
- the iclR gene coding for the regulator in the central intermediary metabolism (Journal of Bacteriology 172: 2642-2649 (1990)),

- the mopB gene coding for the 10 Kd chaperone (WO 03/004669), which is also known under the name groES,
- the ahpC gene from the ahpCF operon coding for the small sub-unit of alkyl hydroperoxide reductase (WO 03/004663),
- the ahpF gene from the ahpCF operon coding for the large sub-unit of alkyl hydroperoxide reductase (WO 03/004663),
- the cysK gene coding for cysteine synthase A (WO 03/006666),
- the cysB gene coding for the regulator in the cys regulon (WO 03/006666),
- the cysJ gene from the cysJIH operon coding for the flavoprotein in NADPH sulfite reductase (WO 03/006666),
- the cysI gene from the cysJIH operon coding for haemoprotein in NADPH sulfite reductase (WO 03/006666),
- the cysH gene from the cysJIH operon coding for adenylylsulfate reductase (WO 03/006666),
- the phoB gene from the phoBR operon coding for the positive regulator PhoB in the pho regulon (WO 03/008606),
- the phoR gene from the phoBR operon coding for the sensor protein in the pho regulon (WO 03/008606),
- the phoE gene coding for protein E in the outer cell membrane (WO 03/008608),
- the pykF gene coding for the pyruvate kinase I stimulated by fructose (WO 03/008609),
- the pfkB gene coding for 6-phosphofructokinase II (WO 03/008610),

- the malE gene coding for periplasmatic binding protein in maltose transport (WO 03/008605),
- the sodA gene coding for superoxidedismutase (WO 03/008613),
- the rseA gene from the rseABC operon coding for a membrane protein with anti-sigmaE activity (WO 03/008612),
- the rseC gene from the rseABC operon coding for a global regulator in the sigmaE factor (WO 03/008612),
- the sucA gene from the sucABCD operon coding for the decarboxylase sub-unit of 2-ketoglutarate dehydrogenase (WO 03/008614),
- the sucB gene from the sucABCD operon coding for the dihydrolipoyltranssuccinase E2 sub-unit of 2-ketoglutarate dehydrogenase (WO 03/008614),
- the sucC gene from the sucABCD operon coding for the β-sub-unit of succinyl-CoA synthetase (WO 03/008615),
- the sucD gene from the sucABCD operon coding for the α-sub-unit in succinyl-CoA synthetase (WO 03/008615),
- the adk gene coding for adenylate kinase (Nucleic Acids Research 13(19): 7139-7151 (1985)),
- the hdeA gene coding for a periplasmatic protein with a chaperonin-like function (Journal of Bacteriology 175(23): 7747-7748 (1993)),
- the hdeB gene coding for a periplasmatic protein with a chaperonin-like function (Journal of Bacteriology 175(23): 7747-7748 (1993)),
- the icd gene coding for isocitrate dehydrogenase (Journal of Biological Chemistry 262(22): 10422-10425 (1987)),

- the mglB gene coding for periplasmatic, galactose-binding transport protein (Molecular and General Genetics 229(3): 453-459 (1991)),
- the lpd gene coding for dihydrolipoamide dehydrogenase (European Journal of Biochemistry 135(3): 519-527 (1983)),
- the aceE gene coding for the E1 component of pyruvate dehydrogenase complex (European Journal of Biochemistry 133(1): 155-162 (1983)),
- the aceF gene coding for the E2 component of pyruvate dehydrogenase complex (European Journal of Biochemistry 133(3): 481-489 (1983)),
- the pepB gene coding for aminopeptidase B (Journal of Fermentation and Bioengineering 82: 392-397 (1996))
- the aldH gene coding for aldehyde dehydrogenase (E.C. 1.2.1.3) (Gene 99(1): 15-23 (1991)),
- the bfr gene coding for the iron storage homoprotein (bacterioferritin) (Journal of Bacteriology 171(7): 3940-3947 (1989)),
- the udp gene coding for uridine phosphorylase (Nucleic Acids Research 17(16): 6741 (1989)) and
- the rseB gene coding for the regulator of sigmaE factor activity (Molecular Microbiology 24(2): 355-371 (1997)).

Furthermore, it may be advantageous for the production of L-amino acids, in particular L-threonine, in addition to overexpressing the galP gene, to attenuate, in particular to switch off or reduce the expression of, one or more of the genes chosen from the group

- the tdh gene coding for threonine dehydrogenase (Journal of Bacteriology 169: 4716-4721 (1987)),
- the mdh gene coding for malate dehydrogenase (E.C. 1.1.1.37) (Archives in Microbiology 149: 36-42 (1987)),
- the gene product of the open reading frame (orf) yjfA (Accession Number AAC77180 at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA, WO 02/29080),
- the gene product of the open reading frame (orf) ytfP (Accession Number AAC77179 at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA, WO 02/29080),
- the pckA gene coding for the enzyme phosphoenolpyruvate carboxykinase (WO 02/29080),
- the poxB gene coding for pyruvate oxidase (WO 02/36797),
- the aceA gene coding for the enzyme isocitrate lyase (WO 02/081722),
- the dgsA gene coding for the DgsA regulator in the phosphotransferase system (WO 02/081721), which is also known under the name mlc gene,
- the fruR gene coding for fructose repressor (WO 02/081698), which is also known under the name cra gene,
- the rpoS gene coding for the sigma^{38}-Factor (WO 01/05939), which is also known under the name katF gene,
- the aspA gene coding for aspartate ammonium lyase (WO 03/008603) und
- the aceB gene coding for malate synthase A (WO 03/008604).

The expression "attenuation" in this connection describes the reduction in or switching off of intracellular activity or concentration of one or more enzymes or proteins in a microorganism, these being coded by the corresponding DNA, by, for example, using a weak promoter or a gene or allele which codes for a corresponding enzyme or protein with a lower activity or by inactivating the corresponding enzyme (protein) or gene and optionally by combining these measures.

Due to the attenuation measures, the activity or concentration of the corresponding protein is generally lowered to 0 to 75%, 0 to 50%, 0 to 25%, 0 to 10% or 0 to 5% of the activity or concentration of the wild-type protein in the starting microorganism.

Furthermore, it may be advantageous for the production of L-amino acids, in particular L-threonine, in addition to overexpressing the galP gene, to switch off undesired side reactions (Nakayama: "Breeding of Amino Acid Producing Microorganisms", in: Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

The microorganisms produced in accordance with the invention may be cultured in a batch process, in a fed batch process or in a repeated fed batch process. A summary of known cultivation methods is given in the textbook by Chmiel (Bioprozesstechnik 1. Einführung in die Bioverfahrenstechnik (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

The culture medium to be used must satisfy the demands of the particular strain in an appropriate manner. Descriptions of culture media for different microorganisms are given in the manual "Manual of Methods for General

Bacteriology" by the American Society for Bacteriology (Washington D.C., USA, 1981).

Suitable sources of carbon which may be used are sugar and carbohydrates such as e.g. glucose, saccharose, lactose, fructose, maltose, molasses, starch and optionally cellulose, oils and fats such as e.g. soy oil, sunflower oil, ground nut oil and coconut fat, fatty acids such as e.g. palmitic acid, stearic acid and linoleic acid, alcohols such as e.g. glycerine and ethanol and organic acids such as e.g. acetic acid. These substances may be used separately or as a mixture.

Sources of nitrogen which may be used are organic nitrogen-containing compounds such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soy bean flour and urea or inorganic compounds such as ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate. The sources of nitrogen may be used separately or as a mixture.

Sources of phosphorus which may be used are phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts. The culture medium also has to contain salts of metals such as e.g. magnesium sulfate or iron sulfate, which are needed for growth. Finally, essential growth substances such as amino acids and vitamins may also be used in addition to the substances mentioned above. Suitable precursors may also be added to the culture medium. The feedstocks mentioned above may be added to the culture in the form of a single mixture or may be fed during cultivation in an appropriate manner.

Fermentation is generally performed at a pH of 5.5 to 9.0, in particular 6.0 to 8.0. Basic compounds such as sodium hydroxide, potassium hydroxide, ammonia and ammonia water or acid compounds such as phosphoric acid or sulfuric acid

are used in an appropriate manner to control the pH. Anti-foam agents such as e.g. fatty acid polyglycol esters can be used to control the formation of foam. Substances which act in a selective manner, such as e.g. antibiotics, can be added to the medium to maintain stability of the plasmids. In order to maintain aerobic conditions, oxygen or oxygen-containing gases, such as e.g. air, are introduced to the culture. The temperature of the culture is normally 25°C to 45°C and preferably 30°C to 40°C. The culture is continued until a maximum of L-amino acids or L-threonine has been produced. This objective is normally achieved within 10 hours to 160 hours.

Analysis of L-amino acids can be performed by anion exchange chromatography followed by ninhydrin derivatisation, as is described in Spackman et al. (Analytical Chemistry 30: 1190-1206 (1958)), or it can be performed by reversed phase HPLC, as is described in Lindroth et al. (Analytical Chemistry 51: 1167-1174 (1979)).

The process according to the invention is preferably used for the fermentative production of L-amino acids such as, for example, L-threonine, L-isoleucine, L-valine, L-methionine, L-homoserine and L-lysine, in particular L-threonine.

Claims

1. A process for the production of L-amino acids by fermentation of recombinant microorganisms of the Enterobacteriaceae family, characterised in that

   a) the microorganisms producing the desired L-amino acid, in which the ga1P gene or nucleotide sequences coding for galactose permease are overexpressed, are cultured in a medium under conditions in which the desired L-amino acid is enriched in the medium or in the cells, and

   b) the desired L-amino acid is isolated, wherein optionally constituents of the fermentation broth and/or the biomass remain in total or in part (> 0 to 100%) in the isolated product or are completely removed.

2. Process according to claim 1, characterised in that recombinant microorganisms are used that are produced by the transformation of a microorganism of the Enterobacteriaceae family with a vector, wherein the vector contains a Ga1p gene.

3. Process according to claim 1, characterised in that in the recombinant microorganisms the copy number of the gene is present increased by at least 1.

4. Process according to claim 3, characterised in that the increase in the copy number of the gene by at least 1 is achieved by integrating the gene into the chromosome of the microorganism.

5. Process according to claim 3, characterised in that the increase in the copy number of the gene by at least 1 is achieved by an extrachromosomally replicating vector.

6. Process according to claim 1, characterised in that in order to achieve the overexpression

   a) the promoter and regulation region for the ribosome-binding site upstream of the ga1P gene is mutated, or

   b) expression cassettes are incorporated upstream of the ga1P gene.

7. Process according to claim 1, characterised in that a ga1P gene is used that is under the control of a promoter.

8. Process according to claim 1, characterised in that by the overexpression of the ga1P gene the activity or concentration of galactose permease is increased by at least 10%, referred to the activity or concentration of the protein in the receptor strain (initial strain).

9. Process according to claim 1, characterised in that microorganisms selected from the genera Escherichia, Erwinia, Providencia and Serratia are used.

10. Process according to claim 1, characterised in that microorganisms are used in which in addition further genes of the biosynthesis pathway of the desired L-amino acid are overexpressed.

11. Process according to claim 10, characterised in that for the production of L-threonine, microorganisms of the Enterobacteriaceae family are fermented, in which in addition one or more of the genes selected from the following group is/are simultaneously enhanced, in particular overexpressed:

   11.1 the thrABC operon coding for aspartate kinase, homoserine dehydrogenase, homoserine kinase and threonine synthase,

11.2 the pyc gene coding for pyruvate carboxylase,

11.3 the pps gene coding for phosphoenolpyruvate synthase,

11.4 the ppc gene coding for phosphoenolpyruvate carboxylase,

11.5 the pntA and pntB genes coding for transhydrogenase,

11.6 the rhtB gene which mediaates homoserine resistance,

11.7 the mqo gene coding for malate:quinone oxidoreductase,

11.8 the rhtC gene which mediates threonine resistance,

11.9 the thrE gene coding for threonine export protein,

11.10 the gdhA gene coding for glutamate dehydrogenase,

11.11 the hns gene coding for DNA binding protein HLP-II,

11.12 the pgm gene coding for phosphoglucomutase,

11.13 the fba gene coding for fructose biphosphate aldolase,

11.14 the ptsH gene coding for phosphohistidine protein hexose phosphotransferase,

11.15 the ptsI gene coding for enzyme I of the phosphotransferase system,

11.16 the crr gene coding for the glucose-specific IIA component,

11.17 the ptsG gene coding for the glucose-specific IIBC component,

11.18 the lrp gene coding for the regulator of the leucine regulon,

11.19 the csrA gene coding for the global regulator Csr,

11.20 the fadR gene coding for the regulator of the fad regulon,

11.21 the iclR gene coding for the regulator of the central intermediary metabolism,

11.22 the mopB gene coding for the 10 Kd chaperone,

11.23 the ahpC gene coding for the small sub-unit of alkyl hydroperoxide reductase,

11.24 the ahpF gene coding for the large sub-unit of alkyl hydroperoxide reductase,

11.25 the cysK gene coding for cysteine synthase A,

11.26 the cysB gene coding for the regulator of the cys regulon,

11.27 the cysJ gene coding for the flavoprotein in NADPH sulfite reductase,

11.28 the cysI gene coding for the haemoprotein in NADPH sulfite reductase,

11.29 the cysH gene coding for adenylylsulfate reductase

11.30 the phoB gene coding for the positive regulator PhoB of the pho regulon,

11.31 the phoR gene coding for the sensor protein of the pho regulon,

11.32 the phoE gene coding for protein E in the outer cell membrane,

11.33 the pykF gene coding for the pyruvate kinase I stimulated by fructose,

11.34 the pfkB gene coding for 6-phosphofructokinase II,

11.35 the malE gene coding for periplasmatic binding protein of maltose transport,

11.36 the sodA gene coding for superoxidedismutase,

11.37 the rseA gene coding for a membrane protein with anti-sigmaE activity,

11.38 the rseC gene coding for a global regulator of the sigmaE factor

11.39 the sucA gene coding for the decarboxylase sub-unit of 2-ketoglutarate dehydrogenase,

11.40 the sucB gene coding for the dihydrolipoyltranssuccinase E2 sub-unit of 2-ketoglutarate dehydrogenase,

11.41 the sucC gene coding for the β-sub-unit of succinyl-CoA synthetase,

11.42 the sucD gene coding for the α-sub-unit of succinyl-CoA synthetase,

11.43 the adk gene coding for adenylate kinase,

11.44 the hdeA gene coding for a periplasmatic protein with a chaperonin-like function,

11.45 the hdeB gene coding for a periplasmatic protein with a chaperonin-like function,

11.46 the icd gene coding for isocitrate dehydrogenase,

11.47 the mglB gene coding for the periplasmatic, galactose-binding transport protein,

11.48 the lpd gene coding for dihydrolipoamide dehydrogenase,

11.49 the aceE gene coding for the E1 component of pyruvate dehydrogenase complex,

11.50 the aceF gene coding for the E2 component of pyruvate dehydrogenase complex,

11.51 the pepB gene coding for aminopeptidase B and

11.52 the aldH gene coding for aldehyde dehydrogenase,

11.53 the bfr gene coding for the iron storage homoprotein,

11.54 the udp gene coding for uridine phosphorylase and

11.55 the rseB gene coding for the regulator of sigmaE factor activity.

12. Process according to claim 1, characterised in that microorganisms are used in which the metabolic pathways that reduce the formation of the desired L-amino acid are partly switched off.

13. Process according to claim 1, characterised in that for the production of L-threonine, microorganisms of the Enterobacteriaceae family are fermented, in which in addition one or more of the genes selected from the following group is/are simultaneously attenuated, in particular switched off or their expression is reduced:

13.1 the tdh gene coding for threonine dehydrogenase,

13.2 the mdh gene coding for malate dehydrogenase,

13.3 the gene product of the open reading frame (orf) yjfA,

13.4 the gene product of the open reading frame (orf) ytfP,

13.5 the pckA gene coding for the enzyme phosphoenolpyruvate carboxykinase,

13.6 the poxB gene coding for pyruvate oxidase,

13.7 the aceA gene coding for isocitrate lyase,

13.8 the dgsA gene coding for the DgsA regulator in the phosphotransferase system,

13.9 the fruR gene coding for fructose repressor

13.10 the rpoS gene coding for the sigma38-Factor,

13.11 the aspA gene coding for aspartate ammonium lyase and

13.12 the aceB gene coding for malate synthase A gene.

14. Recombinant microorganisms of the Enterobacteriaceae family, in particular of the genus Escherichia, in which the galP gene or nucleotide sequences coding for galactose permease are present overexpressed.

15. Microorganisms according to claim 14, characterised in that these produce L-threonine.

16. Process according to claims 1 and 12, characterised in that L-amino acids selected from the group L-asparagine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine are produced.

17. Process according to claims 1 to 10 and 12, characterised in that L-amino acids selected from the group L-isoleucine, L-valine, L-methionine, L-homoserine and L-lysine are produced.

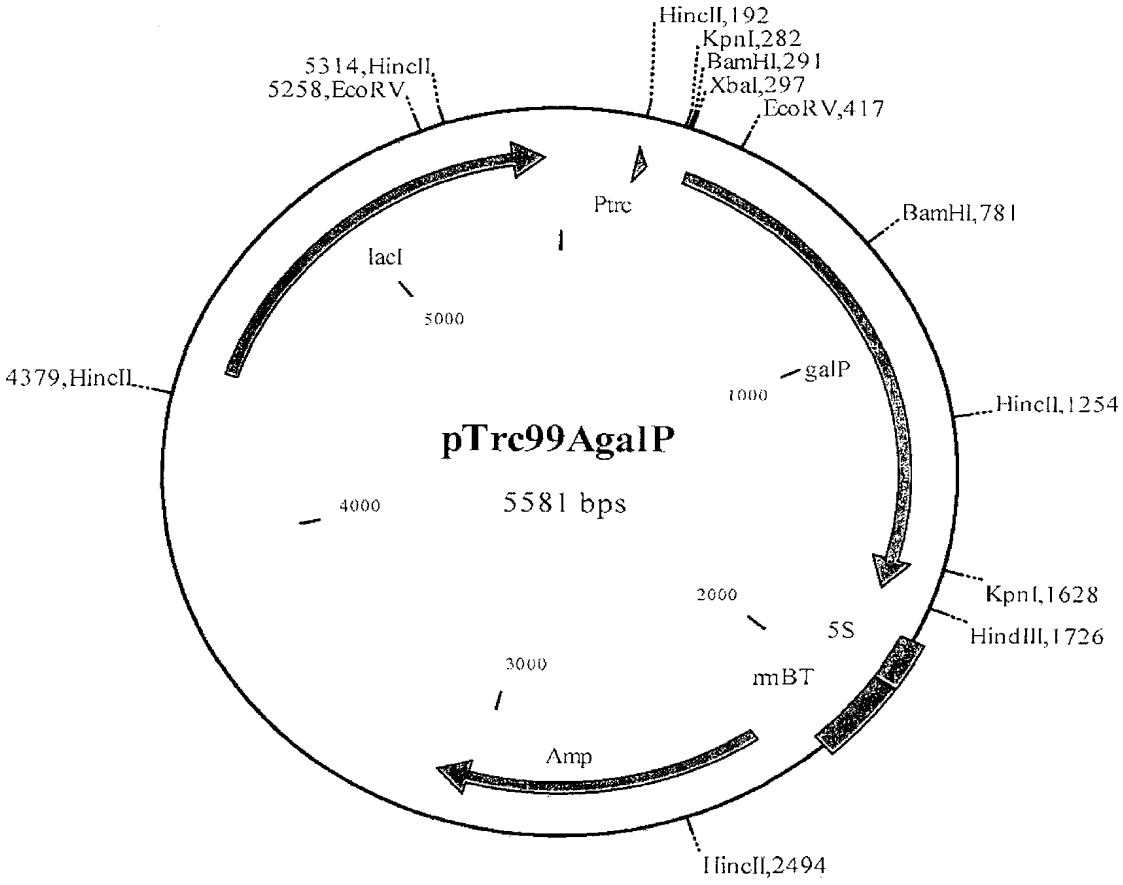
18. Process according to claims 1 to 13, characterised in that L-threonine is produced.

Abstract

The invention relates to a process for the production of L-amino acids by fermentation of recombinant microorganisms of the Enterobacteriaceae family, characterised in that

a) the microorganisms producing the desired L-amino acid, in which the ga1P gene or nucleotide sequences coding for galactose permease is/are overexpressed, are cultured in a medium under conditions in which the desired L-amino acid is enriched in the medium or in the cells, and

b) the desired L-amino acid is isolated, wherein optionally constituents of the fermentation broth and/or the biomass remain in total or in part (> 0 to 100%) in the isolated product or are completely removed.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